

Amendments to the Specification

Please replace the paragraph that spans page 6 lines 29-32 through page 7 lines

1-16 with the following amended paragraph:

To obtain cDNA, total RNA was isolated from the G250 producing hybridoma cells according to the method by Chomczynski et al. (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162 (1987), 156-159) and converted into cDNA essentially as described by Maniatis et al. Amplification of cDNA sequences by PCR is possible only, if the sequence of the gene of interest is known. In general, for PCR two primers complementary to the 5'-end and the 3'-end of the sequence are used as the initiation point of DNA synthesis. Because the sequence of the 5'-ends of the VH and VL chain from the G250 monoclonal antibody producing hybridoma cells were unknown, the PCR method, referred to as RACE (rapid amplification of cDNA ends) was used to amplify the VH and VL chain. This was achieved by employing anchor [SEQ ID No. 1] and anchor-poly-C [SEQ ID No. 2] primers and the constant VH [SEQ ID No. 3] and VL [SEQ ID No. 4] -primers as shown in Fig 2. The VH and VL fragments were purified and ligated into pGEM11 as described by Maniatis et al. A ligation mixture was introduced into bacteria, which were selected and expanded. DNA was isolated from the selected bacterial colonies and analyzed by restriction enzyme digestion to confirm the presence of the amplified VH and VL fragments. Three positive colonies were subjected to DNA sequencing. The sequences of these three individual clones were compared and found to be identical.

Please replace the paragraph on page 7 lines 18-19 with the following amended paragraph:

Portions of the resulting sequences including the antigen-specific CDR regions are shown in Fig. 1 [VH G250: nucleic acid SEQ ID No. 5, amino acid: SEQ ID No. 6; VL G250: nucleic acid SEQ ID No. 7, amino acid SEQ ID No. 8].

Please replace the paragraph on page 8 lines 3-21 with the following amended paragraph:

The G250 VH and VL chain cDNA sequences were obtained as described in co-pending US-patent application 60/327,008, example 3. The resulting cDNA fragments, a 2.3 kb EcoRI heavy chain variable region fragment and a 5.5 kb HindIII light chain variable region fragment were cloned into suitable expression vectors which contain the human G1 constant region (for the H-chain) or the human Kappa constant region (for the L-chain), respectively, and genes conferring resistance to selectable markers. Competent bacteria (E.coli TG1) were transformed with the plasmids. Ampicillin resistant clones were selected and expanded. Plasmid DNA was isolated using the Nucleobond AX 500 Maxiprep Kit from Machery & Nagel (Germany). The isolated DNA was subjected to cycle sequencing using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Freiburg, Germany) and the resulting DNA molecules labeled with multiple fluorescent dyes were analyzed using the ABI PRISM Model 377 DNA Sequencer (Applied Biosystems, Weiterstadt, Germany). The employed sequencing primers are shown in the following. For sequencing of the full length inserts, the 2.3 kb EcoRI and 5.5 kb HindIII, respectively, a primer-walking approach was applied. The obtained Sequences of both the CDR's as well as the heavy and light chain is shown in Fig. 3 [Figure 3A: SEQ ID No. 9; Figure 3B: SEQ ID No. 10].

Please replace the paragraph that spans page 8 lines 23-30 through page 9 lines 1-2 with the following amended paragraph:

Primers used for Cycle Sequencing of the variable region of G250 heavy (H) and light (L) chain

RightH GAG GTT CCT TGA CCC CAG T [SEQ ID No. 11]

LeftH CGA TTC CCA GTT CCT CAC A [SEQ ID No. 12]

RightL AAC GTC CAC GGA TAG TTG CT [SEQ ID No. 13]

LeftL CAG AAC AGC ATG GGC TTC A [SEQ ID No. 14]

The sequencing results are shown in Fig. 3. The primer sequences are underlined.
The CDR sequences are boxed.

Please replace the paragraph on page 19 line 31 with the following amended paragraph:

A summary of the results can be taken from Figure 5 [light chain: SEQ ID No. 15; heavy chain: SEQ ID No. 16].

Please replace the paragraph on page 20 lines 3-5 with the following amended paragraph:

Figure 6 [light chain: SEQ ID No. 17; heavy chain: SEQ ID No. 18] shows the sequence coverage of WX-G250 in the LC-MS/MS experiment of a tryptic digest without reduction and alkylation of the antibody.

Please replace the paragraph on page 20 lines 18-23 with the following amended paragraph:

Tryptic fraction 5 contained the expected sequence E E Q Y ? [SEQ ID No. 19] corresponding to residues 295-298 (hc). The glycosylated N following the Y cannot be seen in Edman sequencing. Together with the peptide mass for peptide 295-303 determined by MALDI-MS it could be proven that this sequence was indeed glycosylated at position 300. Two minor contaminations were also found in this HPLC fraction: VSITC* [SEQ ID No. 20] and LIVSL [SEQ ID No. 21].

Please replace the paragraph on page 20 lines 25-28 with the following amended paragraph:

VSITC* [SEQ ID No. 20] was derived from a Light chain peptide starting at position 19. It contained a Cys modified by iodacetamide. LIVSL [SEQ ID NO. 21] could not be annotated to the WX-G250 structure. It is possible that this peptide was derived from trypsin.

Please replace the paragraph on page 21 lines 1-5 with the following amended paragraph:

LysC fraction 17 was close to the detection limit (< 0.5 pmol) but proved to be the expected sequence: S? G? T? A S V V ? C? L L? [SEQ ID No. 22]. However, due to limited amount of sample it was not possible to sequence to the expected deamidation site which followed the two leucins. But together with the MALDI-MS data the deamidation is evident.

Please replace the paragraph on page 21 lines 7-11 with the following amended paragraph:

LysC fraction 21 clearly showed the expected sequence T K P R E [SEQ ID No. 23] corresponding to residues 291-295 (hc). Together with the peptide mass for peptide 291-319 determined by MALDI-MS it could be proven that this sequence was indeed glycosylated at position 300. This is in accordance with the Edman sequencing result of tryptic fraction 5.